**recomLine HCV IgG**

**IVD**

**Instructions for use (English)**

1 **Purpose**
   The recomLine HCV IgG is a qualitative test for the detection of IgG antibodies against the Hepatitis C Virus (HCV) in human serum or plasma.

2 **Intended use**
   The recomLine HCV IgG is a line immunoassay. The test principle allows for separate lining up of the individual antigens and thus, unlike ELISA, for the identification of specific antibodies against the individual HCV antigens (Core 1, Core 2, Helicase, NS3, NS4, NS5). The recomLine HCV IgG is a confirmation test and can be used to clarify unclear screening results.

3 **Test principle**
   Highly purified recombinant HCV antigens are fixed on nitrocellulose membrane strips.
   1. The test strips are incubated with the diluted serum or plasma sample, and the specific antibodies bind to the pathogen antigens on the test strips.
   2. Unbound antibodies are then flushed away.
   3. In a second step, the strips are incubated with anti-human immunoglobulin antibodies (IgG), which are coupled to horseradish peroxidase.
   4. Unbound conjugate antibodies are then flushed away.
   5. Specifically bound antibodies are detected with a staining reaction catalysed by the peroxidase. If an antigen-antibody reaction has taken place, a dark band will appear on the strip at the corresponding point.

   There are control bands at the upper end of the test strips:
   a) Reaction control is located below the strip number, and needs to show a reaction for each serum/plasma sample.
   b) The conjugate control band (IgG) is used to check the detected antibody class. If, for example, the test strip is used for the detection of IgG antibodies, the IgG conjugate control will show this clearly on the band.
   c) "Cut-off control": The intensity of this band allows the assessment of the reactivity of each antigen band (see 9.2. Evaluation).

4 **Reagents**

4.1 **Package contents**
   The reagents in one package are sufficient for 20 tests.
   - **WASHBUF A** (10X) 100 ml Wash Buffer A (10 times concentration)
   - **SUBS** (10X) 40 ml Chromogenic substrate Tetramethylbenzidine (TMB, ready-to-use)
   - **MICROFLOW** 2 g skimmed milk powder
   - **INSTRU** 1 Instructions for use
   - **EVALFORM** 1 Evaluation form
   - **TESTSTR1** 2 tubes, each with 10 numbered test strips
   - **CONT1** (100 µl positive serum control IgG (hundred times concentrated, green screw cap))
   - **CONT2** (100 µl negative serum control IgG (hundred times concentrated, green screw cap))
   - **TMB**, ready-to-use

4.2 **Materials required but not supplied**
   - Volumetric cylinders, 50 ml and 1000 ml
   - Micropipettes with disposable tips, 20 µl and 1000 µl
   - 10 ml pipette or dispenser
   - Timer
   - Absorbent paper towels
   - Disposable protective gloves
   - Waste container for bio-hazardous materials

5 **Shelf life and handling**
   - Store reagents at +2 °C to 8 °C before and after use, do not freeze.
   - Subject all ingredients for at least 30 minutes to room temperature (+18 °C to 25 °C) before beginning the test. The test procedure is carried out at room temperature.
   - Washing Buffer, Milk Powder, Dilution Buffer, Conjugate and TMB can be interchanged between the different recomLine and recomBlot test systems, if these components carry the same symbols. Consider the shelf life of these components.
   - Mix the concentrated reagents and samples thoroughly before use.
   - Avoid a build up of foam.
   - Open the tube containing the test strip immediately before use to avoid condensation. Leave unused strips in the tube and continue to store at +2 °C to +8 °C (reseat tube tightly, test strips must not become moist before the test).
   - The strips are marked with the serial number, as well as the test date.
   - The packages bear an expiration date. After this has been reached no guarantee of quality can be offered.
   - Protect kit components from direct sunlight throughout the entire test procedure. The substrate solution (TMB) is especially sensitive to light.

6 **Warnings and precautions**
   - **For in vitro diagnostic use only.**
   - All blood products must be treated as potentially infectious.
   - The test strips were manufactured with inactivated whole cell lysates and / or recombinant produced bacterial, viral or parasitic antigens.
   - After the addition of patient or control specimens the strip material must be considered infectious and treated as such.
   - Suitable disposable gloves must be worn throughout the entire test procedure.
   - The reagents contain the anticoagulants and preservatives sodium azide, MIT (methylisothiazolone), o-phenylenediamine and hydrogen peroxide. Avoid contact with the skin or mucous membrane. Sodium azide can form an explosive azide upon contact with heavy metals such as copper and lead azide.
   - All siphoned liquids must be collected. All collecting containers must contain suitable disinfectants for the inactivation of human pathogens. All reagents and materials contaminated with potentially infectious samples must be treated with disinfectants or disposed of according to your hygiene regulations. The concentrations and incubation periods of the manufacturer must be observed.
   - Use incubation trays only once.
   - Handle strips carefully using plastic forceps.
   - Do not substitute or mix the reagents with reagents from other manufacturers.
   - Read carefully the instructions for use prior to the test and then follow the individual steps indicated. Deviation from the test protocol provided in the instructions for use can lead to erroneous results.
7 Sampling and preparation of reagents

7.1 Samples
The sample can be serum or plasma (citrate, EDTA, heparin, CPD), which needs to be separated from the blood clot as soon as possible after sampling so as to avoid haemolysis. Avoid Microbial contamination of the samples. Insoluble substances must be removed from the sample prior to incubation.
The use of heat-inactivated, icteric, haemo lysed, lipemic or turbid samples is not recommended.

Caution!
If the provisions are not made immediately, the sample can be stored for up to 2 weeks at +2 to +8 °C. Prolonged storage of the samples is possible at -20 °C or below. Repeated freezing and thawing of samples is not recommended due to the risk of inaccurate results. Avoid more than 3 cycles of freezing and thawing.

7.2 Preparation of solutions

7.2.1 Preparation of ready-to-use wash buffer A
This buffer is required for serum and conjugate dilution as well as washing stages.

Prior to dilution, the volume of wash buffer A must be determined for the corresponding number of tests.

First, the skimmed milk powder is dissolved in wash buffer A concentrate and then deionised water is added to bring the solution up to the final volume (dilution: 1 + 9). The quantities required for a defined number of test strips are to be mathematically determined according to the following formula (device-specific dead volume is not considered):

\[ \text{Reagent} \times \text{Formula} \times \text{Example: 5 strips} = \text{number of strips} \times 2 + 0.5 \]  

1. Deionised water [ml]
2. Wash buffer A concentrate [ml]
3. Deionised water [ml]
4. Ready-to-use wash buffer A [ml]

Ready to use wash buffer A can be stored at 2 °C – 8 °C for up to 4 weeks. The ready to use wash buffer A is odourless and easily marred.

7.2.2 Preparation of conjugate solutions
The conjugate solution must be prepared just before use. It is not possible to store the ready-to-use conjugate solution.

One part of the conjugate concentrate is diluted with 100 parts of the ready to use wash buffer A (1 + 100). The quantities required for a defined number of test strips are to be calculated according to the following formula:

\[ \text{Reagent} \times \text{Formula} \times \text{Example: 5 strips} = \text{number of strips} \times 20 + 10 \]  

1. Conjugate concentrate [μl]
2. Ready-to-use wash buffer A [ml]

The conjugate quantities are calculated without dead volume. Depending on handling (manual or on a device), please mix additional conjugate for 1 to 3 strips.

8 Test procedure

<table>
<thead>
<tr>
<th>No.</th>
<th>Execution</th>
<th>Note</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Temper all reagents for at least 30 minutes at 18 °C – 25 °C (room temperature) before beginning the test.</td>
<td>The test procedure is carried out at room temperature.</td>
</tr>
<tr>
<td>2</td>
<td>Prepare test strips</td>
<td>Place the strips in 2 ml of ready-to-use wash buffer A. Do not touch the strips with bare hands - use the forceps. The strip number points upward. A well is required in the incubation tray (see 4.2) for each strip. The strips must be completely immersed.</td>
</tr>
<tr>
<td>3</td>
<td>Incubation of samples</td>
<td>a) 20 μl of undiluted sample (human serum or plasma) or control is pipetted onto the test strip for each incubation mixture. (Dilution 1 + 100) b) Incubate for 3 hours with gentle shaking.</td>
</tr>
<tr>
<td>4</td>
<td>Washing</td>
<td>a) Carefully remove the plastic cover from the incubation trays. b) Gently siphon serum dilution from the individual wells. c) Pipette 2 ml of ready to use wash buffer A into each well, wash for 5 minutes with gentle shaking and then siphon off the wash buffer A. Carry out washing stages 8.4a-8.4c three times in all. Avoid cross-contamination. Avoid cross-contamination. The manufacturer's instructions must be followed during automatic processing.</td>
</tr>
</tbody>
</table>

9 Results

Caution!
Please do not use automated interpretation without consideration of the information on interpretation given below.

9.1 Validation – Quality Control
An analysis of the test can be carried out if the following criteria have been fulfilled:

1. Reaction control band (top line) with clearly visible stain, dark band
2. Antibody class (second band): the IgG conjugate control band must show a clear staining
3. Cut-off control (third band): weak, but visible staining

Negative and positive controls are not required for evaluating the test. They may be carried out for internal quality control purposes wherever necessary.

The controls must have the following reactive antigen bands:
- Positive control: Core 1, Core 2, Helicase, NS3, NS4; NS5 may react, but will not necessarily do so.

Negative control: no

9.2 Evaluation
The analysis of the test strips can be visual or computer-assisted - using the test strip analysis software recomScan. The recomScan software is designed to support the evaluation of test strips. Further information and related instructions for the computer-assisted analysis is available on request from MIKROGEN. The following instructions relate to visual analysis.

9.2.1 Assessment of band intensity
1. Note the date and batch number, as well as the detected antibody class, on the attached evaluation form.
2. Enter the sample identification numbers to the evaluation sheet.
3. Now stick the corresponding test strip onto the appropriate fields on the evaluation form using a glue stick. Align the test strip with the reaction control bands along the marked lines. Then use a transparent adhesive tape to attach the test strip to the marked lines (do not tape over the reaction control band!). Sticking the entire test strip down flat using glue or tape can lead to changes in colour.
4. Now identify the bands of the developed test strip on the basis of the printed control strip of the evaluation sheet and enter them to the evaluation sheet. For each corresponding immunoglobulin class, assess separately the intensity of the bands occurring on the basis of Table 1.

| Table 1: Assessment of band intensity in relation to the cut-off band |
|------------------------|-----------------|
| Stain intensity of the bands | Assessment |
| No reaction | +/- |
| Very low intensity (lower than the cut-off band) | + |
| Low intensity (equivalent to the cut-off band) | ++ |
| High intensity (higher than the cut-off band) | +++ |
| Very strong intensity | ++++ |
9.3 Interpretation of test results
The criteria for interpreting the test are to be taken from Table 2.

Table 2: Test interpretation

<table>
<thead>
<tr>
<th>Test result</th>
<th>Criteria</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>• No Antigen ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• NS3, NS4 or NS5 isolated ≥ cut-off</td>
</tr>
<tr>
<td>Borderline</td>
<td>• Core 1 isolated ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• Core 2 isolated ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• Helicase isolated ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• Helicase and one NS protein (NS3, NS4 or NS5) ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• two other random antigens ≥ cut-off</td>
</tr>
<tr>
<td>Positive</td>
<td>• Core 1 and Core 2 ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• Core 1 and a further antigen ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• Core 2 and a further antigen ≥ cut-off or</td>
</tr>
<tr>
<td></td>
<td>• three random antigens ≥ cut-off</td>
</tr>
</tbody>
</table>

10 Limitations of the method - restrictions

- Serological test results must always be considered in the context of other medical assessments of the patient. Therapeutic consequences of the serological findings must always be taken in context with the clinical data.
- A negative test result cannot exclude an infection with the Hepatitis C virus. In the early phase of infection, antibodies may not yet be present or not present in a detectable quantity. If an HCV infection is suspected, a further sample should be taken after two weeks and tested.
- For hemodialysis patients, it may not be possible to detect HCV antibodies despite positive evidence of HCV-RNA (Hninrichsen et al., 2002; Bukh et al., 1993).
- Patients with borderline results should always be tested again after three to four weeks. As an additional precaution, an (RT-)PCR test for the HCV genome is recommended.
- In patients with acute EBV infection isolated NS5 reactivity was observed. Where the medical history is unclear, it is recommended to exclude EBV infection by differential diagnosis.
- No correlation between positive antibody detection and infectiousness is possible.
- Dark test strips: Some patient samples can produce a dark, uniform or patterned staining across the entire nitrocellulose strip (e.g. on sera from patients with milk protein allergies). Various factors in each patient serum are responsible for this. The evaluation of these strips is usually only partly feasible. Thus, "inverse" bands (white bands on dark background), for example, should be evaluated as negative. The respective serum should always be examined using other serological methods.

11 Test performance

11.1 Diagnostic sensitivity

<table>
<thead>
<tr>
<th>recomLine HCV IgG</th>
<th>HCV* (n=419)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>0</td>
</tr>
<tr>
<td>Borderline</td>
<td>1</td>
</tr>
<tr>
<td>Positive</td>
<td>418</td>
</tr>
</tbody>
</table>

sensitivity = (1+418)/419=100%**

* Including samples of the genotypes 1,2,3,4,5 and 6.
** Including one borderline result.

11.2 Seroconversions

Fifteen seroconversion panels with a total of 123 samples were tested with the recomLine HCV IgG in direct comparison with another commercially available confirmation test. In three panels, the recomLine HCV IgG had antibodies against HCV earlier than the comparison test. In three panels, the recomLine HCV IgG only became responsive in a later sample than the comparison test. In the remaining nine seroconversions both confirmatory tests detected HCV antibodies from the same inspection.

11.3 Diagnostic specificity

<table>
<thead>
<tr>
<th>recomLine HCV IgG</th>
<th>Blood donors (n=297)</th>
<th>Clinical samples* (n=229)</th>
<th>Potentially interfering samples** (n=58)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative</td>
<td>291</td>
<td>224</td>
<td>68</td>
</tr>
<tr>
<td>Borderline</td>
<td>6</td>
<td>5</td>
<td>0</td>
</tr>
<tr>
<td>Positive</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Specificity = (291+224)/297=98.0% 224/229-97.8% 68/68=100%

* Samples from patients with other viral hepatitis, recent EBV or CMV infection, autoimmune diseases, HIV, treponema, yellow fever and tick-borne encephalitis infection, pregnant women and routine laboratory samples.
** Lipemic, haemolytic and icteric samples, RF-positive samples, patients with hypergammaglobulinemia.

11.4 Analytical specificity

The analytical specificity is defined as the capacity of the test to precisely determine the antibodies in the presence of potential interference factors in the sample matrix or cross-reactions with potentially interfering antibodies.

a) Interferences: Control studies on potentially interfering factors have shown that anticoagulants (sodium citrate, EDTA, heparin, CPD), haemolysis (up to 1,000 mg/dl haemoglobin), lipaemia, bilirubinaemia (up to 20 mg/dl bilirubin) or three cycles of freezing and thawing do not affect the performance of the test.

b) Cross-reactions: The potential interference of antibodies to other organisms with similar clinical symptoms to an infection with HCV (e.g. EBV CMV, other viral hepatitis) as well as an infection with related pathogens (tick-borne encephalitis virus, yellow fever virus) were examined in control studies. Additionally, conditions that are attributed to atypical activity of the immune system (antinuclear autoantibodies, rheumatoid factor) were tested. There was no proof of any cross-reactivity (see 11.3).

12 Literature

5. Europäischer Konsens zu Hepatitis C, Epidemiologie, Diagnose und Therapie, Deutsches Arzteblatt Heft 50 17.12.99
6. R. S. Roß, S. Vazov, M. Roggendorf: Möglichkeiten und Grenzen der gegenwärtigen Hepatitis C-Virus-Diagnostik. BIOforum 11/98, 697-701

We will gladly send you further literature on the diagnosis of HCV upon request.
13 Explanation of symbols

<table>
<thead>
<tr>
<th>Symbol</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Σ</td>
<td>Content is sufficient for &lt;n&gt; applications</td>
</tr>
<tr>
<td>EVALFORM</td>
<td>Evaluation form</td>
</tr>
<tr>
<td>INSTRU</td>
<td>Instructions for use</td>
</tr>
<tr>
<td>CONT</td>
<td>Contents, includes</td>
</tr>
<tr>
<td>IVD</td>
<td>In vitro test</td>
</tr>
<tr>
<td>LOT</td>
<td>Batch number</td>
</tr>
<tr>
<td>REF</td>
<td>Order number</td>
</tr>
<tr>
<td>x°C to y°C</td>
<td>Store at x°C to y°C</td>
</tr>
<tr>
<td>Manufacturer</td>
<td></td>
</tr>
</tbody>
</table>

14 Manufacturer and version information

recomLine HCV IgG

<table>
<thead>
<tr>
<th>Item no.</th>
<th>4372</th>
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<tbody>
<tr>
<td>Instructions for use</td>
<td>GARLHC005EN</td>
</tr>
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<td>valid from</td>
<td>2014-03</td>
</tr>
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</table>

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